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Contamination and Growth of the Shrimp, *Penaeus stylirostris* Stimpson, Cultured in a Seawater/Wastewater Aquaculture System

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During the past three decades there has been a substantial increase in the consumer's demand for "seafoods". This has led to an increased fishing effort, which, along with man's alteration of shallow water habitats, has resulted in a decrease of natural stocks and an increase in the cost of shellfish and finfish. Possible answers to this increase in demand may be found in either an increased fishing effort, the traditional approach, or in the cultivation of popular marine organisms. However, many commercial mariculture operations have failed because of the costs associated with rearing animals in high density cultures. One of the most significant costs is feed. Ryther et al. (1972) have suggested that the costs of culturing marine animals could be lowered by reducing the stocking densities used in the ponds and substituting nutrient rich treated wastewater for commercial feeds. The increased biological production could support the animals and the system would have the additional advantage of serving as a tertiary water treatment plant (Goldman et al. 1974).

However, an aquaculture system which is based on wastewater may not only have a different production profile than a system based on prepared feeds, but the animals may also be subject to contamination (Furr et al. 1981). We have monitored the growth of shrimp cultured in a seawater/wastewater system and have analyzed water an tissue samples for several likely organic and inorganic contaminants.

MATERIALS AND METHODS

Shrimp were cultured in earthen ponds, 10m X 10m X 0.5m, lined with PVC plastic and plumbed with seawater from the Harbor Branch Foundation ship canal, an

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extension of the Indian River in Fort Pierce, Florida. Two of the ponds (I and II) were also plumbed to receive effluent from the Harbor Branch Foundation secondary treatment plant, so these ponds contained a 10% mixture of effluent. Postlarval (8-11 mg) Penaeus stylirostris Stimpson were released so that two of the ponds, I and IV, were low density cultures containing 4.5 and 6.0 shrimp/m^2, respectively, and two of the ponds, II and III, were stocked at a high density of 30.0 shrimp/m^2. Ponds I and II received effluent while III and IV were fed Purina Experimental Marine Ration LF (25% protein, 5% crude fat, 5% fiber) as follows: when shrimp in III and IV reached an average of 0.5gm they began to receive 10% of their wet weight per day; the absolute amount of feed added to the ponds was then kept constant resulting in a ration that dropped to 2-3% after two months. This 2-3% level was then maintained for the growing shrimp until harvesting. After five months the shrimp were harvested, counted, and 30 were taken as a subsample from each pond to get the mean weights; the heads and carapaces were removed, and the abdominal muscle freeze-dried.

Salinity, temperature, and dissolved oxygen were recorded weekly. Readings were made in the late morning or early afternoon. Salinity was determined using an American Optical temperature compensated refractometer. Temperature and dissolved oxygen were measured using a Yellow Springs Instrument Oxygen Meter model 51 B.

Water samples (950 ml) were examined for organic contaminants once every two weeks for the last two months of the culture period. Water from each of the four ponds was collected in hexane washed glass bottles, acidified to pH 2-3, then extracted three times with 15 ml of hexane. Frozen-dried tissue was spiked with 10 ug each of fluoranthene and n-octodecane then extracted for 8 to 18 hours in a soxhlet apparatus using acetone:hexane:acetic acid (59:40:1). Five shrimp, a reagent blank, and a spiked blank were analyzed. The samples were reduced in volume in vacuo then under N_2; the extracts were then placed on microcolumns of (top to bottom): Na_2SO_4, silica gel, alumina, and glass wool. All materials had been activated in a muffle furnace at 500°C. The column was eluted with five bed volumes of hexane to recover the aliphatic hydrocarbon fraction, followed by toluene to recover the polynuclear aromatic hydrocarbon (PAH) fraction (Pierce et al. 1983). The samples were then reduced to an appropriate volume for

GC and HPLC analysis. Phenols were extracted from 4 organisms from each pond, a reagent blank, and a blank spiked with 1.04 ug pentachlorophenol (PCP). This was done in a soxhlet apparatus for six hours using hexane. The samples were twice washed with H_2O then twice with 0.1N NaOH. The base was then acidified with HCl to pH 2 and extracted three times with hexane. The hexane fraction containing the chlorinated phenols was reduced under N_2 (Pierce 1978).

The phenol and PAH samples were analyzed with a Varian model 5020 HPLC using a MCH-10 (Varian Instruments, Sunnyvale, California) reverse phase column with a mobile phase of methanol:water:acetic acid (90:9:1) (v/v). The compounds were detected at a UV wavelength of 254 nm with a Varian UV-50 spectrophotometer. Secondary analysis of PAHs was performed by gas chromatography using a Varian model 6000 gas chromatograph coupled to a Vista-401 data system. Columns were glass capillary (SE-30, 30m X 0.25mm), and the carrier gas was N_2. Samples were introduced by direct inject splitless injection mode, and temperature programmed from 100° to 280° at 8°C/minute.

Water samples to be analyzed for heavy metals collected when the other water samples were taken. Water was taken in duplicate from ponds II (effluent pond) and III (control pond) in polyethylene bottles washed with warm, 2 to 4 N nitric acid. The samples were taken to the laboratory and filtered through acid washed 0.45um polycarbonate Nuclepore filters. Cadmium, chromium, copper and lead were coprecipitated with colbalt- ammonium pyrollidine dithiocarbamate (Boyle and Edmond 1975). A spiked sample containing 1 ug Cd, Cr, Cu and Pb was also prepared to adjust for extraction efficiency. Wet tissue was analyzed by General Activation Analysis, Inc. (San Diego, CA 92121 USA) for Se using high flux neutron activation analysis (Lucas *et al.* 1970). Tissues to be examined for the other four metals were digested in tall form beakers covered with watch glasses using warm HNO_3 with complete oxidation resulting from the addition of H_2O_2.

Water and tissue sampled for Cd, Cr, Cu and Pb were analyzed with a Perkin-Elmer model 460 atomic absorption spectrophotometer using a HGA-400 graphite atomizer, an AS-40 autosampler, and a deuerium-arc background corrector.

Results from the analysis of the water and tissue samples were compared using the nonparametric

Table 1. Survival, mean weights ± standard deviation, and total yields.

Pond	Stocking Density	Percent Survival	Mean Weight ± standard deviation (gm)	Projected Yield (Kg) per hectare
I- Effluent	Low	29	16.2 ± 5.11	210
II- Effluent	High	27	3.9 ± 1.14	320
III- Feed	High	42	4.7 ± 1.41	590
IV- Feed	Low	50	14.8 ± 5.00	430

Wilcoxon-U test and growth was tested with a one-way ANOVA coupled with Scheffe's equations for multiple comparison (Wonnacott and Wonnacott 1977). Pond parameters which varied with ambient conditions were compared using sets of randomly chosen pairs subjected to a sign test for matched samples (Schefler 1979).

RESULTS AND DISCUSSION

Survival rates, mean weights and total yields are shown in Table 1. *P. stylirostris* grown in low density cultures were significantly larger ($P<0.001$) than those grown in high density. There was no significant difference in the size of the shrimp from ponds I and IV ($P>0.20$) although those from pond III were larger than those from pond II ($0.01<P<0.05$).

Oxygen levels in the ponds were adequate (3.2 to 11.4 ppm), temperatures ranged from 22° to 36°C, and salinities from 16.3 o/oo to 34.6 o/oo. Treatment ponds were statistically indistinguishable from control ponds with respect to D.O. and temperature: however, the ponds receiving the wastewater consistently had lower salinities, by about 2 o/oo, than did those receiving only seawater. This difference was significant ($P<0.05$).

Water samples taken on three of the four sampling days contained less than 10 ug/1 PAH and phenol compounds; one water sample contained several small unidentified peaks, and subsequent analysis using GC-FID showed no difference between the control and test ponds. The hydrocarbon extracts of the shrimp samples exhibited similar gas chromatographic patterns indicative of biogenic material with no major apparent differences between the control ponds and the test ponds receiving treated sewage effluent. No petroleum hydrocarbon patterns were observed in either the aliphatic or aromatic (PAH) fractions. These results were supported by the HPLC-UV analysis which showed the internal

Table 2. Mean heavy metal concentration ± standard deviation in *Penaeus stylirostris* and in water samples.

Element	Pond	ug/gm shrimp dry weight)	ug/liter pond water
Cd	I	0.27 ± 0.32	
Cd	II	0.18 ± 0.11	0.055 ± 0.009
Cd	III	0.12 ± 0.06	0.081 ± 0.025
Cd	IV	0.13 ± 0.08	
Cr	I	0.99 ± 0.16	
Cr	II	0.89 ± 0.33	0.95 ± 0.60
Cr	III	0.73 ± 0.18	0.99 ± 0.43
Cr	IV	0.94 ± 0.29	
Cu	I	32.1 ± 6.91	
Cu	II	26.9 ± 7.86	32.08 ± 12.91
Cu	III	31.3 ± 4.75	30.29 ± 20.81
Cu	IV	30.3 ± 6.26	
Pb	I	0.07 ± 0.02	
Pb	II	0.09 ± 0.04	0.03 ± 0.02
Pb	III	0.11 ± 0.03	0.04 ± 0.03
Pb	IV	0.03 ± 0.01	
Se	I	0.94 ± 0.23	
Se	II	1.38 ± 0.19	
Se	III	1.35 ± 0.29	
Se	IV	1.13 ± 0.18	

standards (PAHs) and very little other UV-absorbing material. The phenol extracts showed a considerable amount of UV-absorbing material corresponding with PCP and other later eluting peaks. PCP quantitation was hindered by excessive interference; however, the patterns were similar for all four ponds showing no qualitative or quantitative differences between control and test ponds. Attempts to remove the interfering compounds by trimethylsilane with subsequent column chromatographic clean up proved unsuccessful.

Heavy metal data are summarized in Table 2. Cadmium may have been concentrated by shrimp in the effluent treated ponds ($0.05<P<0.10$). Both lead ($0.001<P<0.01$) and selenium ($P=0.02$) were found in higher titers in the small *Penaeus*. No other differences were found in either the water or tissue samples.

Although Penaeus stylirostris can be mass cultured using sewage effluent as the sole nutrient source, the biomass produced is less than that when a commercial feed is used. Pond I yielded on 47% as much shrimp as Pond IV, and Pond II 54% as much as pond III. The exact reasons are not obvious but may be related to the amount of food available, the quality of the commercial feed pellets as opposed to the algae and associated fauna, or it may be a function of water quality. Stocking density and/or final density was inversely proportional to the mean weight of the shrimp. Similar observations with regard to stocking density and growth have been made for P. aztecus (Venkataramaih and Lakshmi 1972) and for P. monodon and P. orientalis (Forster and Beard 1974).

While the total yield of P. stylirostris was somewhat disappointing from a commercial standpoint, the results are more encouraging when viewed in light of what others have found using different species. P. stylirostris growth was appreciably better than the growth of other members of this genus found locally on the east coast of Florida which have been reared under conditions similar to those described in this report. Williams and Ryther (unpublished) reared P. duorarum and P. setiferus in 14% effluent from post larvae to the juvenile stage (3-6 gm), but continued growth was very slow. The shrimp with the lowest growth rates (Pond II) increased in weight as quickly as the local species, P. aztecus and P. duorarum, reared under optimal conditions with prepared feed (Subrahmanyam and Oppenheimer 1971).

Chlorinated phenols have been found in domestic sewage (Buhler et al. 1973) and low levels ($\leq$0.5 ug/g) were observed in oysters grown in the Indian River (Van Gelder, 1982). Aquatic organisms quickly absorb these compounds upon exposure, yet depuration is rapid when exposure ceases (Kobayashi and Akitake 1975). The fact that no PCP could be detected in the water, but peaks corresponding to PCP were found in the chromatograms of all the shrimp extracts, suggests that if PCP did enter the system, it came from the ship canal rather than from the sewage, and was either below our detection limit (<10 ug/l) or came in only periodically and quickly entered either the shrimp directly or via the pond detritus. Polycyclic aromatic hydrocarbons are widely distributed in the aquatic environment (Basu and Saxena 1978), although in situ levels may vary considerably. The results in this report are similar to those of Mann et al. (1979) who grew Crassostrea gigas in wastewater, and showed that

the oyster also did not concentrate hydrocarbons or PCBs. However, Landau (1983) showed that the effluent from the HBF treatment plant was relatively dilute compared to most municipal operations, and therefore this report should not be taken to mean the P. stylirostris will not concentrate these organics if more are present in the treated wastewater.

There is an extensive body of data on the concentration of metals in marine invertebrates, but little that applies to these experiments. Lobsters cultured in wastewater (Furr et al. 1981) seemed to concentrate more metals than did the shrimp reared in the HBF ponds. The concentrations of the metals in the cultured P. stylirostris fell close to or within the ranges reported for various species of shrimp in Sidwell's review (1981). Boyden (1974) examined populations of six species of estuarine molluscs for heavy metals. He found in some species there was no correlation between body weight and metal concentration, while other species concentrated more of certain metals when the organism was small; only one limpet concentrated more of one element as it grew. It is therefore not suprising that two of the five metals were more concentrated in the shrimp from ponds II and III while the others metals showed no such relationship. It is not possible at this time to predict which animals will concentrate which metals. Sadiq et al. (1982) found no correlation between the size of P. semisulcatus and Cd, Cr, Cu, or Pb.

The levels of the dissolved metals in the ponds, with the exception of Cu, were considerably lower than expected based on previous work done in the coastal ocean (Abdullah et al. 1972, Smith et al. 1981). However, there was a relatively dense suspension of material in the ponds, so much so that during the preparation of the water samples the filters constantly became obstructed so that filtering 250 ml would take from two to three hours. O'Connor and Kester (1975) have shown that adsorption of several metals will readily occur on suspended materials. We suspect that our low metal values are a result of loss by filtration. This is supported by work on the Danish coast by Magnusson and Rasmussen (1982) who also reported low values and who used filtration techniques similar to ours. The high levels of Cu are probably from antifouling paints from vessels in the ship canal; two large ships which are painted regularlyare moored 200-400 m from the intake pipe bringing water into the system.

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